# CEPHALOTHIN

## Its In Vitro Antibacterial Spectrum as Studied in a Diagnostic Laboratory

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■ *Of 655 bacterial strains isolated in a routine clinical diagnostic laboratory, 564 (86 per cent) were found sensitive to cephalothin by disk sensitivity test. However, the fraction of sensitive Gram-negative bacilli and enterococci declined sharply at concentrations approximating those obtained in serum after the usual recommended dosages. Gram-positive bacteria remained sensitive throughout the entire range of testing concentrations.*

*Unless very high dosages are used, cephalothin cannot be considered a broad-spectrum antibacterial agent for use while cultures are pending. Even with very high dosages all strains of* Pseudomonas spp. *and significant fractions of other Gram-negative bacillary species remain resistant to cephalothin.*

*If used alone, the high potency cephalothin disk may yield inadequate information and should be supplemented by a lower potency disk, standardized measurements of zone diameters, or a screening plate.*

A NEW antimicrobial, cephalothin (Keflin®, Lilly), is bactericidal, effective against Gram-positive and Gram-negative bacterial species *in vitro* and *in vivo*, devoid of inherent toxicities and resistant to degradation by staphylococcal penicillinase.[1,3,5,9] Cephalothin is chemically related to the penicillins, but does not produce crossed allergic hypersensitivity reactions. Minor, thus far reversible, cutaneous hypersensitivity reactions, eosinophilia and transient neutropenia have been reported.

The potential usefulness of cephalothin in the treatment of infections due to Gram-positive cocci and some Gram-negative bacteria prompted a comparison of the *in vitro* sensitivity-resistance patterns of bacteria isolated in a routine diagnostic laboratory to cephalothin and other antimicrobials.

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## Materials and Methods

All specimens submitted for culture and antibacterial sensitivity determinations to the Infectious Disease Laboratory at the Palo Alto-Stanford Hospital Center between 21 December 1964 and 11 February 1965 were processed with plate screening tests. No attempt was made to control inoculum size.

Primarily to test Gram-positive cocci, plate concentrations of 1.0 unit per ml for penicillin-G and 2 mcg per ml for erythromycin were chosen to approximate the concentrations attained in serum after commonly employed dosages. Each ampicillin, neomycin, streptomycin, tetracycline and chloramphenicol plate contained 10 mcg per ml, and each colistin plate 5 mcg per ml, to approximate *peak* serum concentrations after full dosages.

Each isolate was also tested against the commercially available 30 mcg cephalothin disk (Baltimore Biological Laboratories). This concentration is very high compared to the serum concentrations achieved after usual dosages of cephalothin.[6] Three cephalothin screening plates were employed to avoid difficulties in comparing results obtained by the disk method with those from the plate screening technique. One plate contained 5 mcg per ml to approximate the mean concentration anticipated after the recommended dosage of 0.5 gm intramuscularly.[4,6] The second contained 10 mcg per ml to approximate the *peak* concentrations with 0.5 gm intramuscularly.[4,6] The third plate contained 20 mcg per ml to approximate *peak* serum concentrations attained after the administration of 1 gm intramuscularly.[4,6] Similar and even higher concentrations are achievable after intravenous therapy.[6]

Susceptibility was determined by absence of growth on the screening plates or by any zone of inhibition around the 30 mcg cephalothin disk after incubation of the plates at 37°C for 16 to 18 hours.

To assess further the *in vitro* sensitivities of various microorganisms to cephalothin, all strains resistant to 20 mcg per ml by the plate screening technique, but sensitive to the 30 mcg disk under the conditions described, were evaluated by the tube dilution method. One tenth ml of a 1:100 dilution of a 24-hour broth culture of the test organism was added to a series of tubes containing 2.0 ml of brain heart infusion broth and cephalothin in concentrations of 5, 10, 20, 30, 40, and 100 mcg per ml. The minimum inhibitory concentration (MIC) was recorded as the lowest concentration of cephalothin required to inhibit growth after 24 hours of incubation at 37°C. These microorganisms were further studied by repeating the plate screening and disk studies, using standard heavy inocula. The blood agar plate surface was streaked once with a cotton swab from an undiluted overnight broth culture of the test organism.

## Results

The laboratory received 3,168 specimens originating from diverse clinical sources; 655 were subjected to sensitivity testing. The microorganisms tested and their sensitivity patterns to the various antimicrobials are shown in Tables 1 and 2.

Of the 655 isolates, 563 (86 per cent) were sensitive to the 30 mcg cephalothin disk, with similarly wide ranges exhibited only by chloramphenicol (85 per cent) and neomycin (81 per cent).

TABLE 1.—*Sensitivity of 655 Bacterial Isolates to Cephalothin*

| Bacterial Species | No. of Strains | 30 mcg disk | | Plate Screening Tests | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 20 mcg/ml | | 10 mcg/ml | | 5 mcg/ml | |
| | | No. | Per Cent * | No. | Per Cent | No. | Per Cent | No. | Per Cent |
| Staph. aureus | 194 | 194 | 100.0 | 193 | 99.5 | 193 | 99.5 | 193 | 99.5 |
| Staph. albus | 64 | 64 | 100 | 64 | 100 | 64 | 100 | 64 | 100 |
| Nonhem. strepto. | 37 | 37 | 100 | 37 | 100 | 36 | 97 | 36 | 97 |
| Pneumococcus | 4 | 4 | 100 | 4 | 100 | 4 | 100 | 4 | 100 |
| Hemolytic strepto. | 14 | 14 | 100 | 14 | 100 | 14 | 100 | 14 | 100 |
| Enterococci | 16 | 15 | 94 | 13 | 81 | 4 | 25 | 3 | 19 |
| Diphtheroids | 6 | 6 | 100 | 6 | 100 | 6 | 100 | 6 | 100 |
| Bacillus spp. | 1 | 1 | 100 | 1 | 100 | 1 | 100 | 1 | 100 |
| Total Gram-positive | 336 | | 99.7 | | 98.8 | | 95.8 | | 95.5 |
| Escherichia coli | 138 | 115 | 83 | 75 | 54 | 55 | 40 | 30 | 22 |
| Klebsiella-Aero. | 47 | 32 | 68 | 25 | 53 | 16 | 34 | 13 | 28 |
| Pseudomonas | 37 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Proteus (Indol+) | 25 | 21 | 84 | 20 | 80 | 20 | 80 | 14 | 56 |
| Proteus (Indol−) | 30 | 26 | 87 | 23 | 77 | 17 | 57 | 11 | 37 |
| Paracolon | 10 | 9 | 90 | 5 | 50 | 2 | 20 | 1 | 10 |
| Salmonella | 1 | 1 | 100 | 1 | 100 | 1 | 100 | 0 | 0 |
| Herellea | 3 | 1 | 33 | 0 | 0 | 0 | 0 | 0 | 0 |
| Providence, Mima, Serratia, Bacteroides | 8 | 3 | 38 | 1 | 13 | 1 | 13 | 1 | 13 |
| Neisseriae | 20 | 20 | 100 | 20 | 100 | 17 | 85 | 17 | 85 |
| Total Gram-negative | 319 | | 71.5 | | 53.3 | | 40.4 | | 27.3 |
| Total bacterial isolates | 655 | | 86.0 | | 76.8 | | 69.0 | | 64.0 |

* If fractions not included, per cent rounded off to nearest integer.

TABLE 2.—*Sensitivity of 655 Bacterial Isolates to Various Antimicrobials*

| Bacterial Species | No. Str. | Pen-G | | P-50* | | Strepto. | | Tetra. | | Chloro. | | Erythro. | | Neomycin | | Colistin | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No. | Per Cent | No. | Per Cent | No. | Per Cent | No. | Per Cent | No. | Per Cent | No. | Per Cent | No. | Per Cent | No. | Per Cent |
| Staph. aureus | 194 | 88 | 45 | 108 | 55 | 150 | 77 | 153 | 79 | 188 | 97 | 181 | 93 | 189 | 97 | 0 | 0 |
| Staph. albus | 64 | 24 | 37 | 41 | 64 | 47 | 73 | 34 | 53 | 58 | 91 | 59 | 92 | 64 | 100 | 0 | 0 |
| Nonhem. strep. | 37 | 37 | 100 | 37 | 100 | 0 | 0 | 27 | 73 | 37 | 100 | 37 | 100 | 0 | 0 | 0 | 0 |
| Pneumococcus | 4 | 4 | 100 | 4 | 100 | 0 | 0 | 4 | 100 | 4 | 100 | 4 | 100 | 0 | 0 | 0 | 0 |
| Hem. strep. | 14 | 14 | 100 | 14 | 100 | 0 | 0 | 10 | 71 | 14 | 100 | 14 | 100 | 0 | 0 | 0 | 0 |
| Enterococci | 16 | 0 | 0 | 16 | 100 | 0 | 0 | 5 | 31 | 16 | 100 | 15 | 94 | 0 | 0 | 0 | 0 |
| Diphtheroids | 6 | 6 | 100 | 6 | 100 | 5 | 83 | 6 | 100 | 6 | 100 | 5 | 83 | 6 | 100 | 0 | 0 |
| Bacillus spp. | 1 | 0 | 0 | 0 | 0 | 1 | 100 | 1 | 100 | 1 | 100 | 1 | 100 | 1 | 100 | 1 | 100 |
| Total Gram-positive | 336 | | 51.5 | | 67.3 | | 60.4 | | 71.4 | | 96.4 | | 94.0 | | 77.4 | | 0.3 |
| E. coli | 138 | 0 | 0 | 85 | 62 | 108 | 78 | 104 | 75 | 130 | 78 | 0 | 0 | 135 | 98 | 78 | 57 |
| Kleb.-Aero. | 47 | 0 | 0 | 5 | 11 | 33 | 70 | 37 | 78 | 35 | 75 | 0 | 0 | 44 | 94 | 24 | 51 |
| Pseudomonas | 37 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 5 | 0 | 0 | 0 | 0 | 3 | 8 | 30 | 81 |
| Prot. (Ind.+) | 25 | 0 | 0 | 21 | 84 | 21 | 84 | 2 | 8 | 19 | 76 | 0 | 0 | 25 | 100 | 0 | 0 |
| Prot. (Ind.−) | 30 | 0 | 0 | 24 | 80 | 25 | 83 | 0 | 0 | 17 | 57 | 0 | 0 | 28 | 93 | 3 | 10 |
| Paracolon | 10 | 0 | 0 | 6 | 60 | 8 | 80 | 9 | 90 | 9 | 90 | 0 | 0 | 9 | 90 | 5 | 50 |
| Salmonella | 1 | 0 | 0 | 1 | 100 | 1 | 100 | 1 | 100 | 1 | 100 | 1 | 100 | 1 | 100 | 0 | 0 |
| Herellea | 3 | 0 | 0 | 0 | 0 | 3 | 100 | 2 | 67 | 0 | 0 | 0 | 0 | 3 | 100 | 3 | 100 |
| Providence, Mima, Serratia, Bacteroides | 8 | 0 | 0 | 0 | 0 | 1 | 13 | 3 | 38 | 2 | 25 | 1 | 13 | 7 | 88 | 1 | 13 |
| Neisseriae | 20 | 8 | 40 | 20 | 100 | 18 | 90 | 18 | 90 | 20 | 100 | 11 | 55 | 18 | 90 | 2 | 10 |
| Total Gram-negative | 319 | | 2.5 | | 50.8 | | 68.3 | | 55.8 | | 73.0 | | 4.1 | | 85.6 | | 45.8 |
| Total bacterial isolates | 655 | | 27.6 | | 59.2 | | 64.3 | | 63.8 | | 84.9 | | 50.2 | | 81.4 | | 22.4 |

* Ampicillin

See text for concentrations of antimicrobials.

Fractions rounded off to nearest integer.

When lower concentrations of cephalothin were investigated, only 418 (64 per cent) of all isolates were inhibited at 5 mcg per ml and 451 (69 per cent) at 10 mcg per ml. These figures are comparable to those for ampicillin (59 per cent), streptomycin (64 per cent) and tetracycline (64 per cent).

When only Gram-positive bacterial species were examined, all concentrations of cephalothin tested yielded greater than 95 per cent inhibition, results similar to those obtained with chloramphenicol (96 per cent) and erythromycin (94 per cent). Cephalothin was more efficacious than ampicillin against staphylococci, which accounted for two-thirds of the Gram-positive isolates, while ampicillin was more effective against enterococci. Penicillin-G inhibited only 52 per cent of the Gram-positive bacteria. Staphylococci and enterococci accounted for the greatest fraction of Gram-positive cocci that were resistant to penicillin-G.

The sensitivity of Gram-negative bacilli to cephalothin diminished sharply as the test concentration declined. Of the 319 Gram-negative bacterial isolates, 71 per cent were sensitive to the 30 mcg disk, 54 per cent to the 20 mcg per ml, and 41 per cent to the 10 mcg per ml, but only 27 per cent to the 5 mcg per ml concentration. Of the same strains, 51 per cent were sensitive to 10 mcg per ml of ampicillin, which was slightly more effective than cephalothin at comparable concentrations. In declining order, neomycin, chloramphenicol, streptomycin and tetracycline were also more effective than cephalothin against Gram-negative bacilli. The overall effectiveness of colistin fell between the 10 and 20 mcg per ml concentrations of cephalothin and below those of neomycin, chloramphenicol, streptomycin, tetracycline and ampicillin. However, colistin inhibited 30 (81 per cent) of 37 Pseudomonas strains against which all of the other antimicrobials were essentially ineffective.

Ampicillin was more effective than cephalothin against *E. coli* and *Proteus mirabilis*. Only the 30 mcg cephalothin disk showed greater efficacy against these two bacterial species than did ampicillin. Cephalothin was somewhat more effective than ampicillin against members of the *Klebsiella-Aerobacter* group.

Of the 61 strains found to be resistant to the 20 mcg per ml screening plate, but sensitive to the 30 mcg disk of cephalothin, 37 were studied by the tube dilution method. The results are shown in Table 3.

TABLE 3.—*Tube Dilution Studies of Strains Resistant to 20 mcg per ml Screening Plate and Sensitive to 30 mcg Disk.*

| | Cephalothin Minimum Inhibitory Concentration (mcg/ml) | | | | | | |
|---|---|---|---|---|---|---|---|
| *Organism* | *5* | *10* | *20* | *30* | *40* | *>100* | *Total* |
| E. coli | 1 | 0 | 8 | 13 | 2 | 1 | 25 |
| Klebsiella-Aerobacter | 1 | 0 | 2 | 0 | 1 | 2 | 6 |
| Paracolon | 0 | 0 | 1 | 1 | 0 | 0 | 2 |
| Proteus mirabilis | 0 | 0 | 0 | 0 | 2 | 1 | 3 |
| Enterococcus | 0 | 0 | 0 | 1 | 0 | 0 | 1 |

On repeat examinations with controlled inocula, 28 of the 37 strains yielded the same results as when the inoculum size was not controlled; four were resistant to the 30 mcg disk, and for all of these the MIC was more than 100 mcg per ml; and five were sensitive to the 20 mcg per ml but resistant to the 10 mcg per ml screening plate. The MIC was 30 mcg per ml for four of these strains and 40 mcg per ml for the fifth strain.

## Discussion

Cephalothin is a valuable addition to the therapeutic armamentarium. However, it is not the universally effective agent so often desired in complicated instances of hospital-acquired infection and superinfection commonly encountered in patients whose defense mechanisms may be seriously compromised by disease or therapy. Currently its main role appears to be in the treatment of penicillin-G resistant staphylococcal infections in patients allergic to the penicillins.

The data presented show that cephalothin possesses a wide range of antibacterial activity. However, this requires qualification. Of 655 diverse strains isolated from a variety of clinical sources involving most organs and tissues of the body, 563 (86 per cent) were inhibited by the 30 mcg cephalothin disk. Cephalothin inhibited all staphylococci regardless of their susceptibility to penicillin-G, as did methicillin (data not included in this report). At the highest test concentration, cephalothin also inhibited all but one of 16 strains of enterococci.

Significant fractions of *Proteus spp., Klebsiella-Aerobacter,* and some other Gram-negative bacilli were resistant to 30 mcg of cephalothin, but only *Pseudomonas spp.* remained completely resistant. The anti Gram-negative bacillary efficacy of cephalothin declined sharply with test concentrations approaching those obtained in body fluids after commonly employed dosages.[6,8]

If cephalothin were used as a broad spectrum agent pending the results of culture, intramuscular or intravenous doses several times those ordinarily recommended would be required to assure adequate coverage against many Gram-negative bacilli.[6] Even then, all infections due to *Pseudomonas spp.*, many due to *Klebsiella-Aerobacter,* and almost 20 per cent of *E. coli* infections would probably not be covered.

Results from the plate screening technique were comparable to those obtained by tube dilution. Of the 37 strains resistant to the 20 mcg per ml screening plate, 24 (65 per cent) had an MIC by tube dilution of more than 20 mcg per ml and 32 (86 per cent) had an MIC of more than 10 mcg per ml.

A close correlation also existed between the disk and tube dilution techniques. Of the 37 strains initially reported sensitive to the 30 mcg disk and resistant to the 20 mcg per ml screening plate, four were resistant to the disk on repeated examinations using the standard heavy inocula. According to the techniques employed in this laboratory, sensitivity to the 30 mcg cephalothin disk probably indicates an MIC of less than 40 mcg per ml by tube dilution.

The 30 mcg disk should probably be supplemented with either a lower potency disk, standardized measurements of zone diameters or a screening plate to offer better correlations between concentrations achieved following the usual recommended dosage schedules and *in vitro* testing. In this laboratory, the 10 mcg per ml screening plate serves the purpose, as it has with ampicillin.

To date there appears to be no dose-related toxicity attributable to cephalothin other than pain at injection sites and thrombophlebitis.[6] In this respect it is potentially superior as a broad spectrum agent to neomycin and chloramphenicol, and also to ampicillin which is ineffective against the penicillin-G resistant staphylococci. Ampicillin also shares crossed allergenicity with other penicillins.

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